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Prevalence and Distribution of Plant-Parasitic Nematodes Associated with Doum Palm Trees *Hyphaene thebaica* (L.) Mart. in Aswan Southern Egypt with Emphasis on Biochemical and Molecular Identification of Root-Knot Nematode

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ABSTRACT

An intensive field study was carried out during the period from December, 27th 2016 to November, 3rd 2017 to survey nematode associations of doum palm trees, *Hyphaene thebaica* in Aswan governorate, Southern Egypt. In this regard, a total of 105 composite rhizosphere soil and root samples were collected from different provinces (Aswan, Daraw, Edfu and Kom Ombo) belonging to Aswan governorate. Nematodes were extracted by Cobb's wet-sieving and centrifugal sugar flotation techniques and identified to the genus level according to the original body descriptions and standard identification keys of plant-parasitic nematodes. Fourteen genera were found to be associated with the rhizosphere of doum palms and could be descendly arranged based on their frequency of occurrence (FO%) as follows: *Meloidogyne* (46.7%), *Rotylenchulus* (33.3%), *Helicotylenchus* (27.6%), *Aphelenchus* (17.1%), *Tylenchus* (14.3%),*Hemicriconemoides* (12.4%), *Tylenchorhynchus* (9.5%), *Ditylenchus* (8.6%), *Aphelenchoides* (7.6%), *Pratylenchus* (6.7%), *Trichodorus* (5.7%), *Criconemella* (4.8%), *Paratylenchus* (3.8%) and *Hoplolaimus* (2.9%). It was clearly noticed that root-knot (*Meloidogyne*), reniform (*Rotylenchulus*) and spiral (*Helicotylenchus*) nematodes appeared to be the most prominent genera recording high prominence value (PV) reaching 3444, 2816 and 1902, respectively and population density (PD) 504, 488 and 362 nematodes/250 cc^3 soil, consequently, whereas the rest genera were less prominent representing PV ranging between 109-595 with PD reaching 46-201 nematodes/250 cc^3 soil. Examination of root samples revealed the presence of ideal root galling caused by *Meloidogyne* and their sedentary adult females were isolated and subjected to the traditional identification to the species level using perineal pattern test and reconfirmed by the biochemical and molecular identification methods. *Meloiodogyne javanica* appeared to be the predominant root-knot nematode species in this study. The current results updated the database of nematode associations with their plant hosts in Egypt and introduced doum palm as new host of *M. javanica* and probably to other nematodes.

Key words: *Hyphaene thebaica,* plant-parasitic nematodes, *Meloidogyne javanica*, esterase isozyme phenotypes, molecular identification, PCR.

INTRODUCTION

Plant-parasitic nematodes (PPN) are of great significance as real soil pests threatening plant production in Egypt and throughout the world. They are commonly associated with the rhizosphere soils of different species of agricultural crops as well as palm trees and can damage plant roots not only through direct feeding and migration within plant tissues, but they also facilitate subsequent root infestation by secondary pathogens, such as fungi and bacteria. Although many PPN have been recorded to be associated with most of the economic crops and horticultural plants, root-knot nematodes (RKN), *Meloidogyne* spp. are the most important and destructive ones. Symptoms associated with RKN infection include root galling, shoot chlorosis, stunted growth and nutrient deficiencies (Luc et al., 2005; Hunt and Handoo, 2009; Abd-Elgawad and Askary, 2015).

Information concerning the occurrence, densities and distribution of PPN associating with different types of palm trees such as date, coconut, oil and ornamental palms are available (Ismail and Eissa, 1993; Youssef and Eissa, 1994; Guevara et al., 1995; Ibrahim et al., 2000; Al-Yahya et al., 2001; Abu-Gharbieh and Al-Azzeh, 2004; Griffith et al., 2005; Mani et al., 2005; Eissa et al., 2009; El-Sherbiny, 2011; Youssef, 2014). However, nothing is mentioned in the literature about the nematode associations of doum palm trees so far.

Doum palm, *Hyphaene thebaica* (L.) Mart. (Family: Arecaceae) is commonly found in southern Egypt and widely planted in Aswan governorate (Täckholm, 1974). It is a dioecious tree growing up to 10-17 m in height. It is easily recognizable by the dichotomy of its stem, where trunk is distinguished by Y-shaped which divided into two branches, each branch is divided again into two branches, and the ends of the branches contain tufts of large leaves (Fig. 1). The female palm produces edible oval woody fruits that persist on the tree for a long time (Orwa et al., 2009; El-Beltagi et al., 2018). Doum fruits contain high levels of essential minerals such as potassium, sodium, calcium, magnesium, and phosphorus. As well as, they contain B-complex vitamins, carbohydrates and fibers which are essential for good nutrition of humans (Admassu et al., 2013). Potential therapeutic properties of doum fruits were documented in the literature. Their beverage traditionally is used for treatment of hypertension, bilharzias, and hematuria bleeding and their aqueous extracts contains high levels of phenols and flavonoids, and possess significant antioxidant and anticancer activities (Hsu et al., 2006), anti-diabetic properties (Salib et al., 2013), anti-inflammatory activity (Shalaby and Shatta, 2013), antibacterial activity towards *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Erwinia carotovora* (Dosumu et al., 2006; Moawad and Abd EL-Rahman, 2014) and antifungal activity against *Aspergillus niger*, *Microsporum gypseum*, *Trichloro phytonrubrum*, *Mucor* sp., *Fusarium solani* and *Candida albicans* (Irobi and Adedayo, 1999).

Unfortunately, doum palm didn't receive interest from nematologists as yet. Therefore, the objectives of present survey study were to identify and analyze the community of the PPN genera associated with doum palm plantations intensively grown in Aswan governorate, southern Egypt. However, information about the occurrence, population densities and distribution of PPN associating with doum palm is of great importance for analyzing their potential to cause economic damage and for suggesting

plans of protection and strategies of management of nematodes on this plant. On the other hand, there is a need for updating the database of PPN status and their new host plants in Egypt.

Figure 1. A doum palm plantation (right) with a close view of their fruits (left) grown in Fares region belonging to Kom Ombo province, Aswan governorate, southern Egypt.

MATERIALS AND METHODS

1- Nematode sampling and extraction:

During the period from December, 27th 2016 to November, 3rd 2017, a total of 105 composite rhizosphere soil and root samples of the doum palm trees widely planted in Aswan governorate, southern Egypt were collected to survey PPN genera. Survey locations including: Aswan botanical garden (Aswan province), Al-Grewat , Kilh Sharq, El-Hassaya, Kilh El-Doumareya (Edfu province), Fares region (Kom Ombo province) and El-Raqabah-Benban (Daraw province). Soil and associated root samples (approximately 1 kg, each) were carefully gathered using a field hoe and stainless steel shovel, 50–75 cm away from the base of the palm trees and up to a 20–40-cm depth from at least 3 sites around the tree after removing the upper soil layer (5-10 cm) because nematodes cannot survive in soil due to the extremely environmental conditions, placed in labeled plastic bags and maintained in cold sampling box till sending to the Nematology Laboratory at the Integrated Protection Laboratory, Plant Protection Research Station, Sabahiya, Alexandria in order to proceed nematode extraction (Coyne et al., 2007). Each composite soil sample was thoroughly mixed in its plastic bag, and nematodes were extracted from a 250 cm^3representative sub sample by method of Cobb's wet-sieving and centrifugal sucrose flotation techniques (Ayoub, 1980). Sieves used in nematode extraction were U.S. standard sieve series of 20, 100, and 400 meshes with pore opening diameters of 850, 150, and 38 μ, respectively.

2- Nematode identification and community analysis:

Nematode suspensions were freshly examined and identified to the genera level based on original descriptions of body characteristics of females and juvenile forms illustrated by Goodey (1963), Mai and Lyon (1975) and periodicals of the Commonwealth Institute of Helminthology (C.I.H.) Descriptions of PPN were examined by Optika B-130 microscope binocular at 10x magnification, and their population densities were recorded using 1-ml Peter's eelworm counting slide.

Analysis of nematode communities including the frequency of occurrence (FO%), population density (PD) and prominence value (PV) were estimated according to Norton (1978), where FO%.= number of positive samples containing a genus/total number of collected soil samples X 100, PD = number of nematode individuals/250 cm^3 soil, and $PV = PD\sqrt{FO\%}$.

3- Identification of root-knot nematodes (RKN):

3.1. Perineal patterns:

For examination of root galling caused by RKN, root samples of doum palm were gently washed free of adhering soil particles, stained for 15-20 min in an aqueous solution of Phloxine B (0.15 g L^{-1} water) and then rewashed with tap water to remove the residuals of staining in order to clarify egg masses of the RKN in red color (Daykin and Hussey, 1985). Sedentary adult females of the RKN were finely isolated from the infected galled roots using a fine needle and their posterior ends (10 specimens) were cut and immerged in a 45% aqueous solution of lactic acid (v/v) to easily removal of all adhering tissues, mounted in a drop of glycerin on a microslide, cover slipped using nail polish and examined under a stereomicroscope (Taylor and Netscher, 1974). Characteristics of the perineal patterns were described according to Eisenback et al., (1981).

Adult females of the RKN were furtherly subjected to reconfirm species identification using certain biochemical and moleular methods.

3.2. Biochemical identification:

3.2.1. The root-knot nematode culture:

Healthy tomato seedlings (*Solanum lycopersicom* Mill.) cv. Super Strain B (40 days-old with 3-4 true leaves) as susceptible host to the most common RKN species, *Meloidogyne arenaria, M .javanica* and *M. incognita* (Ibrahim et al., 2014) were transplanted in three clean 30-cm diameter clay pots containing mixed remains of *Meloidogyne*-infested soil samples of doum palm (one/pot) and maintained for 60 days under outdoor conditions during the period from March 29th to May 27th, 2017. Eventually, galled tomato roots were excised from potted soil, gently washed by running tap water, wiped between layers of facial tissues, kept in the refrigerator at 4-5ºC in clean plastic bags and sent to the Central Laboratory of Biotechnology, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt for the biochemical and molecular identification of RKN.

3.2.2. Esterase isozyme:

Esterase isozyme phenotyping is the routine diagnostic test for RKN in many Nematology laboratories throughout the world. It is based on the relative mobility of enzymes extracted from mature females on gel electrophoresis (Blok and Powers, 2009). The whole procedure takes three to four hours from sample processing to gel revelation. Protein extract from *M. javanica* females is applied on the gel for use as reference phenotype. Method in details on the can be found in Carneiro and Almeida, (2001). Adult females of the RKN were excised from tomato roots and individually macerated in 0.1 phosphate extraction buffer (pH 7.4) with 20% sucrose, 2% Triton X-100, and 0.1% bromophenol blue dye. Electrophoresis of macerated individual females was done with an automated apparatus PhastSystem™ (Pharmacia, Uppsala, Sweden) on 10 to 15% gradient polyacralamide gels. Esterase phenotypes were determined by staining polyacralamide gels with the substrate α-naphthyl acetate as mentioned by Esbenshade and Triantaphyllou (1990).

3.3. Molecular identification:

Fifty viable *Meloidogyne* females were extracted from the tomato roots by teasing with a fine needle and placed into 2-ml Eppendorf tubes. Females were treated with 0.5% sodium hypochlorite (NaOCl) for 1 min and centrifuged at 3,000 rpm and stored at -80ºC until DNA extraction (Zein et al. 2011).

3.3.1. DNA extraction:

DNA of the adult females of RKN isolated from galled tomato roots was extracted according to the technical protocol tips of CTAB (Cetyl Trimethyl Ammonium Bromide) adapted from Winnepenninckx et al. (1993). Females were powdered in liquid nitrogen in 2-ml eppendorf tubes and 700 ml of extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA and 1% ß-mercaptoethanol was added at the time of utilization. The homogenate was incubated in a water bath for 2 hr at 65ºC, cooled on ice, and spun at 1,000 rpm for one min at 4ºC in a microcentrifuge. The supernatant was transferred to a new tube and an equal volume of chloroform+isoamyl alcohol (24:1) added and extracted twice. After centrifugation at 13,000 rpm for 15 min, approximately 500 ml of the aqueous phase was transferred to a new tube, and DNA was precipitated by adding 2 volumes of isopropanol.The suspension was maintained at 4ºC overnight. After a new centrifugation at 13,000 rpm for 10 min, the supernatant was discarded, and the precipitate was rinsed once with 500 ml of 70% ethanol, air dried for 2 hr, and resuspended in 100 ml TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) plus 40 mg/ml of RNAase. The solution was maintained at 37ºC for 30 min. and incubated in a water bath for 20 min at 60ºC. DNA was reprecipitated by adding 2/3 volume of isopropanol. Microtube was left to precipitate for 2 hr to overnight at room temperature, then centrifuged at13,000 rpm for 15 min at 4ºC to pellet the DNA. The supernatant was carefully removed and the pellet then washed with cold 70% ethanol, air dried for 2 hr, resuspended in 100 ml TE buffer and stored at -20°C (Zein et al., 2011).

3.3.2. Sequence characterized amplified regions (SCARs) amplification and analysis:

In the last few decades, several species-specific primer sets have been developed for *Meloidogyne* diagnosis. Primer design is crucial for the success of this approach. Species-

specific primers must cover any intra-specific variation and not to amplify non-target nematodes. Several primers designed for identification of tropical *Meloidogyne* species are based on SCARs (Sequence Characterized Amplified Regions), including *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *M. paranaensis*, *M. exigua* and *M. enterolobii*. SCAR markers are developed from the characterization and sequencing of polymorphic bands resulting from random amplified polymorphic DNA (RAPD) analysis (Cunha et al., 2018). Examples of specific primers for *M .javanica* which we used in this study are shown in (Table 1).

Table 1. Primers used for the molecular identification of *Meloidogyne javanica*.

Name of primer	Primer sequences
Fjav	GGTGCGCGATTGAACTGAGC
Rjav	CAGGCCCTTCAGTGGAACTATAC

The amplification reactions for SCARs were performed in 25 µl reaction volumes containing 2 µl of genomic DNA 60 ng, 2.5 µl of 10x reaction buffer, 2.5 µl of 25 mM $MgCl_2$, 0.75 µl of 2.5 mM dNTPs, 0.5 µl of 25 pmol primers Fjav/Rjav, and 0.5 µl of 1.25 units of Taq DNA polymerase (from Promega Biotechnology Company, USA). The total volume was completed to 25 µl using sterile distilled water. The PCR was conducted using Biometra T-Gradient Thermal Cycler (GmbH, Germany). The thermocycler was programmed for 2 min at 94 ºC followed by 35 cycles of 30 s at 94 ºC, 30 s at the annealing temperature and 1 min at 72 ºC. Annealing temperature was 64 ºC using the primers Fjav/Rjav (Zijlstra et al., 2000). Five µl aliquots + 3 µl dye were taken from the reaction and subjected to the electrophoresis on a 1% agarose gel. Finally, products were visualized by staining with Ethidium bromide (Zein et al., 2011).

RESULTS AND DISCUSSION

Fourteen nematode genera were found to be associated with the rhizosphere of doum palm plantations in Aswan, southern Egypt (Table 2). They could be descendly arranged based on their frequency of occurrence (FO%) as follows: *Meloidogyne* (46.7%), *Rotylenchulus* (33.3%), *Helicotylenchus* (27.6%), *Aphelenchus* (17.1%), *Tylenchus* (14.3%), *Hemicriconemoides* (12.4%), *Tylenchorhynchus* (9.5%), *Ditylenchus* (8.6%), *Aphelenchoides* (7.6%), *Pratylenchus* (6.7%), *Trichodorus* (5.7%), *Criconemella* (4.8%), *Paratylenchus* (3.8%) and *Hoplolaimus* (2.9%).

Most of these genera have also been previously found to be associated with the rhizosphere soil and infecting roots of date and ornamental palm species in Egypt and throughout the world (Ismail and Eissa, 1993, Ibrahim et al., 2000; Al-Yahya et al., 2001, Abu-Gharbieh and Al-Azzeh, 2004, Mani et al., 2005; Ibrahim and Mokbel, 2009; El-Sherbiny, 2011 and Youssef, 2014).

Root galling and nematode egg masses were clearly observed on the feeder roots of doum palm. Moreover, browning, necrosis and distortion of epidermal and cortical tissues were also obviously seen. Root galls were generally varied in size and the small ones mostly accompanied with numerous egg masses (Fig. 2). This observation is in good

agreement with that given by Al-Yahya et al.(2001) on the date palm roots infected with *M. javanica* in Riyadh region, Saudi Arabia.

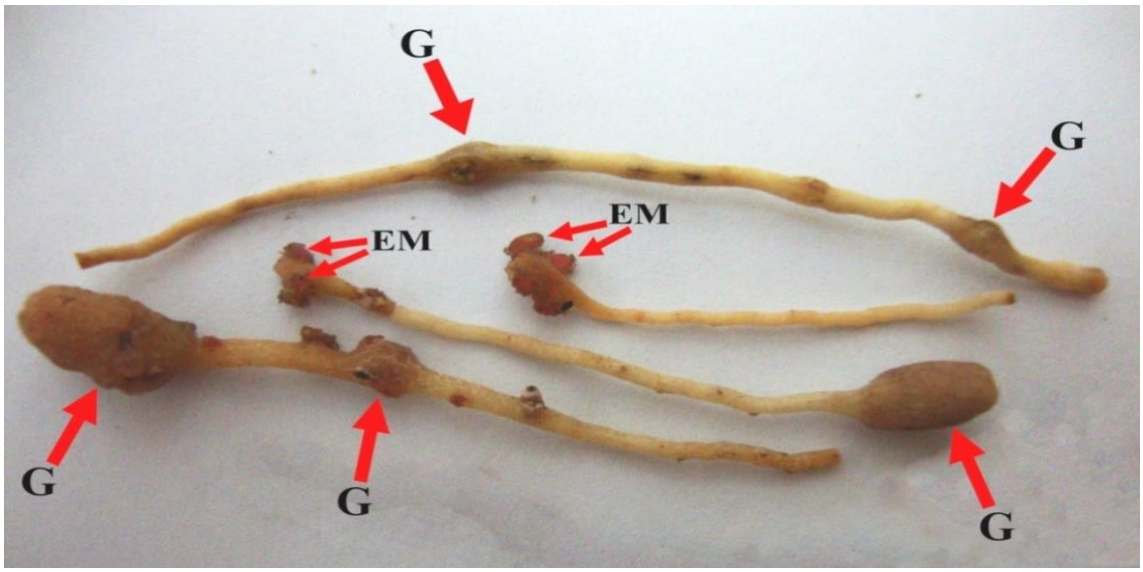

Figure 2. A view of feeder roots of doum palm naturally infested with *Meloidogyne javanica* in Aswan, showing root galls (G) and nematode egg masses (EM) in red following staining with Phloxine B.

According to the calculated prominence values (PV) of all nematode genera, it was clearly noticed that the root-knot (*Meloidogyne*), reniform (*Rotylenchulus*) and spiral (*Helicotylenchus*) nematodes appeared to be the most prominent ones representing PV = 3444, 2816 and 1902, respectively, and PD = 504, 488 and 362 individuals/250 cc^3 soil, consequently, whereas the rest genera were found to be less prominent recording PV ranking between 109-595 and low PD reaching to 46-201 individuals/250 cc^3 soil.

Information about importance of most nematode genera in this study was reported by several authors. Root-knot and reniform nematodes are considered ones of the most damaging genera on numerous host plants as well as palm trees (Abu-Gharbieh and Al-Azzeh, 2004; Griffith et al., 2005; Eissa et al., 2009 ; El-Sherbiny, 2011). Root damage caused by the RKN on palm trees was recorded by some authors. *M. javanica* can severely damage or kill date palm seedlings in greenhouse studies and that date palms also showed a remarkable capacity for quick and abundant root regeneration, even from roots that were extensively galled and rotted (Carpenter, 1964). In addition, high population of *Meloidogyne* species was also recorded in association with diseased date palm trees in Saudi Arabia (Al-Khoury, 1989). The damage of spiral nematode species (*Helicotylenchus*) is usually insidious rather than dramatic and that only four of the almost 190 species of the genus have been consistently associated with plant growth suppression (Yeates and Wouts, 1992).

The lesion nematodes (*Pratylenchus*) are very important migratory endoparasitic nematodes infecting many plant crops and are known to form disease complexes with many different soil-borne fungi causing root rot, thereby increasing root damage. Also, ring nematodes (*Criconemella* and *Hemicriconemoides*) have been considered as important factors limiting the growth of several perennial plants (Sikora and Fernández,

1990). However, *Aphelenchus* and *Tylenchus* spp. are mostly feeding on fungi and lower plants in the soil (Sasser, 1989).

Table 2. Frequency of occurrence (FO %), mean population density (PD) per 250 cm^3 soil, population range (PR), prominence value (PV) and distribution of plant-parasitic nematodes associated with doum palm trees (*Hyphaene thebaica*) in Aswan governorate, Southern Egypt.

Nematode Genera	No. positive samples	FO (%)	PD± SD	PR	PV	Location(s)
Aphelenchoides	8	7.6	50 ± 14	30 - 75	138	Al-Grewat, Aswan botanical garden, El-Raqabah-Benban, Fares.
Aphelenchus	18	17.1	56 ± 13	34 - 80	232	Al-Grewat, Kilh Sharq, El-Raqabah, Benban, Fares, Kilh El-Doumareya.
Criconemella	5	4.8	157 ± 48	119- 240	344	El-Raqabah-Benban, Fares, Kilh Sharq.
Ditylenchus	9	8.6	100± 50	51- 198	293	El-Hassaya, Kilh Sharq, El-Raqabah-Benban, Fares.
Helicotylenchus	29	27.6	362 ± 260	130 -1491	1902	Al-Grewat, Aswan botanical garden, El-Hassaya, Kilh Sharq, El-Raqabah-Benban, Fares, Kilh El-Doumareya.
Hemicriconemoides	13	12.4	146 ± 49	105 - 272	514	Al-Grewat, Aswan botanical garden, KilhSharq, Fares, Kilh El-Doumareya.
Hoplolaimus	3	2.9	64 ± 20	45 - 85	109	KilhSharq, Fares.
Meloidogyne J_2*	49	46.7	504 ± 269	225- 1764	3444	Aswan botanical garden, El-Hassaya, Kilh Sharq, El-Raqabah-Benban, Fares, Kilh El-Doumareya.
Paratylenchus	4	3.8	201 ± 32	162 - 238	392	Kilh Sharq.
Pratylenchus	7	6.7	116± 30	64 - 144	300	Kilh Sharq, Kilh El-Doumareya.
Rotylenchulus	35	33.3	488 ± 233	195 - 1273	2816	Al-Grewat, Aswan botanical garden, Kilh Sharq, El-Raqabah-Benban, Fares, Kilh El-Doumareya.
Trichodorus	6	5.7	46 ± 9	38 - 60	110	El-Hassaya, Fares.
Tylenchorhynchus	10	9.5	193 ± 102	112 - 464	595	Al-Grewat, Kilh Sharq, El-Raqabah-Benban, Kilh El-Doumareya.
Tylenchus	15	14.3	130 ± 26	98- 180	492	Al-Grewat, Aswan botanical garden, El-Hassaya, Kilh Sharq, El-Raqabah-Benban, Fares, Kilh El-Doumareya.

* J_2=second stage juveniles.

FO% = (number of positive samples containing a genus ÷ number of total samples) ×100.

PD = mean number of individuals of a particular genus ÷ number of positive samples.

$PV = PD\sqrt{FO}\%$.

Based on morphological characteristics of the perineal patterns of adult RKN females isolated from doum palm roots, *M. javanica* (Treub) Chitwood was identified and appeared to be dominant RKN species in all surveyed locations in this study. It is characterized by presence of the lateral lines (Fig. 3) in the middle of the pattern which distinguishes this species from other *Meloidogyne* species.

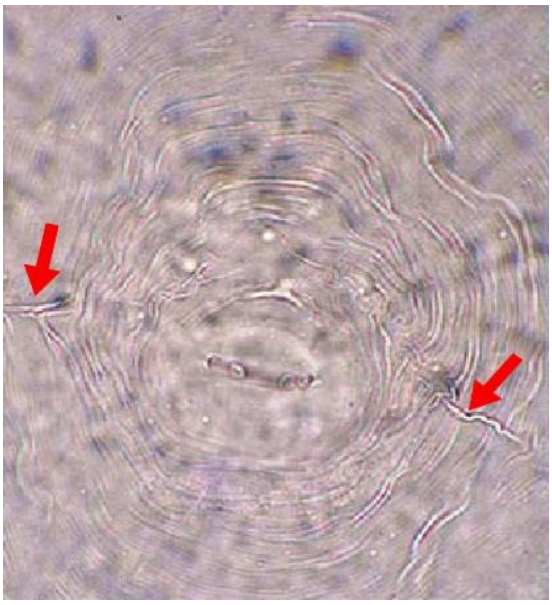

Figure 3. Perineal pattern of *Meloidogyne javanica* (Treub) Chitwood isolated from doum palm roots showing clear lateral lines (arrows).

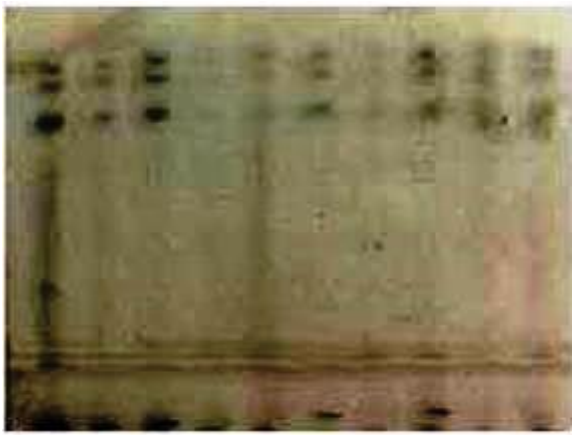

Figure 4. Esterase isozymes identified from individual *Meloidogyne* females following the electrophoresis on polyacrylamide gel.

Data of esterase phenotype identification showed that the common RKN species in this study is the javanese root-knot nematode, *Meloidogyne javanica* (Fig. 4). Fortunately, the used specific primers positively reacted for *M. javanica* as shown in Fig. (5) .These results reconfirmed identification of the RKN species in the present study and greatly agree with those given by Zijlstra et al. (2000).

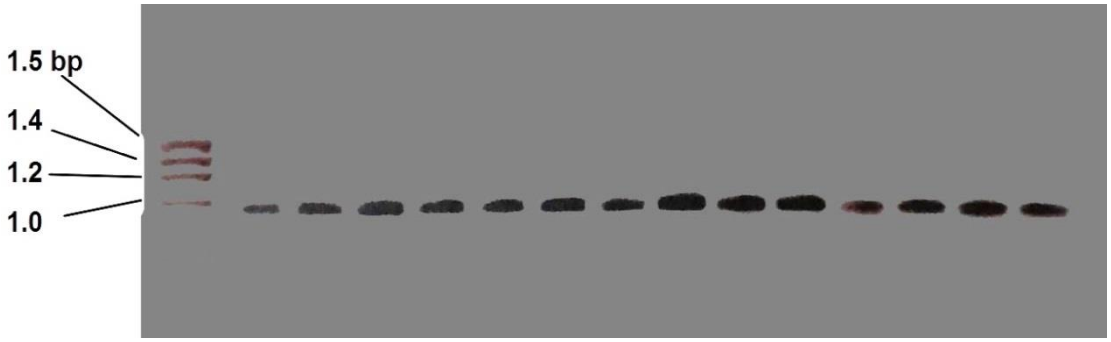

Figure 5. Typical amplification products of PCR reaction for samples of *Meloidogyne javanica.*

The present study introduced doum palm as a new host plant of the Javanese root-knot nematode, *M. javanica* and probably to other PPN genera in southern Egypt. Because of a lack of such documents on the actual relationship between the associated nematodes and diseased doum palm trees, therefore, further studies are still needed to correlate the association of these nematode genera with health problems of doum trees in southern Egypt in order to propose plans of protection and strategies of management of nematode threats.

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REFERENCES

Abd-Elgawad, M.M.M. and Askary, T.H. (2015). Impact of phytonematodes on agriculture economy. In: Biocontrol Agents of Phytonematodes. Askary, T.H. and Martinelli, P.R.P. (eds). CAB International. Pp.3-49.

Abu-Gharbieh, W. and Al-Azzeh, T. (2004). A checklist on nematode-plant associations in the Arab countries. Arab J. Plant Protect. 22 (1): 1-22.

Admassu, M.; Bekele, A. and Kim, J.C. (2013). Nutritional composition of *Balanites aegyptiaca* (desert date) and *Hyphaene thebaica* (doum palm) fruits consumed by Hamadryas Baboons (Papio hamadryas hamadryas) in Awash National Park, Ethiopia. J. Nutritional Ecol. Food Res.1 (3): 198-206.

Al-Khoury, I. (1989). Population dynamics of soil nematodes associated with wijamed and healthy date palms in Al-Hassa Oasis, Saudi Arabia. Pp. 491-507 *in* Proceedings of the Second Symposium on date palm. March 3-6, Date Palm Research Center, Saudi Arabia.

Al-Yahya, F.A.A.; Ibrahim, A.A.M. and El-Sherbiny, A.A.I. (2001). Frequency and population density of plant parasitic nematodes associated with date palm cultivars in Riyadh, Saudi Arabia. Alexandria J.Agric. Res. 46 (2): 127-137.

Ayoub, S.M. (1980). Plant Nematology: an Agriculture Training Aid. Nema/Aid Publications, Sacramento, CA, p. 195.

Blok, V.C. and Powers, T.O. (2009). Biochemical and molecular identification. In: Perry, N.R.; Moens, M. and Starr, J.L. (eds). Root-knot nematodes. Wallingford: CABI Publishing. p.98-118.

Carneiro, R.M.D.G. and Almeida, M.R.A. (2001). Electrophoresis technique used in the study of root-knot nematode enzymes to identify species Nematologia Brasileira 25 (1):35-44.

Carpenter, J.B. (1964). Root-knot nematode damage to date palm seedlings in relation to germination and stage of development. Date Growers Institute Report 41:10-14.

Coyne, D.L.; Nicol, J.M. and Claudius-Cole, B. (2007). Practical Plant Nematology: A field and laboratory guide. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin.

Cunha, T.G.da.; Visôtto, L.E.; Lopes, E.A.; Oliveira, C.M.G. and God, P.I.V.G. (2018). Diagnostic methods for identification of root-knot nematodes species from Brazil. Ciência Rural 48: (2): 1-11.

Daykin, M.E. and Hussey, R.S. (1985). Staining and histopathological techniques in nematology. In: Baker, K.R., Carter, C.C. and Sasser, J.N. (Eds.), In: An Advanced Treatise on *Meloidogyne*. Methodology, Raleigh, Vol. II. North Carolina state University Graphics, pp. 39-48.

Dosumu, O.O., Nwosu, F.O. and Nwogu CD. (2006). Antimicrobial studies and phytochemical screening of extracts of *Hyphaene thebaica* Linn. Mart fruits. Int. J.Trop. Med. 1 (4): 186-189.

Eisenback, J.D.; Hirschmann, H.; Sasser, J.N. and Triantaphyllou, A.C. (1981). A guide to the four most common species of root-knot nematodes (*Meloidogyne* spp.), with a pictorial key. A Cooperative Publication of the Department of Plant Pathology and Genetics, North Carolina State University and USAID, Raleigh, North Carolina, USA.

Eissa, M.F.M.; El-Sherif, M.A.; Abd-El-Gawad, M.M.; Ismail, A.E. and El-Nagdi, W M.A. (2009). Seasonal population dynamics, horizontal and vertical distributions of phytonematodes in date-palm cv. Samani in Egypt. Plant Pathol. Bull. 18: 45-50.

El-Beltagi, H.S.; Mohamed, H.I.; Yousef, H.N. and Fawzi, E.M. (2018). Biological activities of the doum palm (*Hyphaene thebaica* L.) extract and its bioactive components. In: Anwar et al., (eds.). Antioxidants from Natural Sources. IntechOpen.

El-Sherbiny, A.A. (2011). Phytoparasitic nematodes associated with ornamental shrubs, trees and palms in Saudi Arabia, including new host records. Pakistan J.Nematol. 29 (2):147-164.

Esbenshade, P.R. and Triantaphyllou, A.C. (1990). Isozyme phenotypes for the identification of *Meloidogyne* species. J.Nematol. 22 (1):10–15.

Goodey, J.B. (1963). Soil and fresh water nematodes. London, Methuen & Co., LTD, New York: John Wiley & Sons, Inc. 544 pp.

Griffith, R.; Giblin-Davis, R.M.; Koshy, P.K.and Sosamma, V.K. (2005). Nematode parasites of coconut and other palms. In: *Plant parasitic nematodes in subtropical*

and tropical agriculture. The 2nd edition. Luc, M., Sikora R.A. and Bridge J. (Editors). C.A.B. International, Oxon, UK. Pp. 493-527.

Guevara, L.A.; Nieto, L.E. and Agudelo, F.V.de. (1995). Nematodes associated with oil palm, *Elaeis guineensis* Jacq, infected with rot disease. Fitopatol. Colomb. 19 (2): 36-42.

Hsu, B.; Coupar, I.M. and Ng, K. (2006). Antioxidant activity of hot water extract from the fruit of the doum palm, *Hyphaene thebaica*. Food Chem. 98 (2): 317–328.

Hunt, D. and Handoo, Z.A. (2009). Taxonomy, identification and principal species. In: Perry, R.N., Moens, M. and Starr, J.L. (eds). Root-knot Nematodes. Wallingford: CABI Publishing: p. 55–97.

Ibrahim, I.K.A. and Mokbel, A.A. (2009). Occurrence and distribution of the root-knot nematodes *Meloidogyne* spp. and their host plants in northern Egypt. Egyptian J. Experim. Biol. (Botany) 5: 125–129.

Ibrahim, I.K.A.; Handoo, Z.A. and El-Sherbiny, A.A. (2000). A survey of phytoparasitic nematodes on cultivated and non-cultivated plants in Northwestern Egypt. Suppl. J.Nematol. 32 (4S):478–485.

Ibrahim, I.K.A.; Mokbel, A.M. and Hammad, S.E. (2014). Host suitability of some solanaceous plant cultivars to the root-knot nematodes, *Meloidogyne* spp. Glo.Adv. Res. J.Agric. Sci. 3 (5): 136-140.

Irobi, O.N. and Adedayo, O. (1999). Antifungal activity of aqueous extract of dormant fruits of *Hyphaene thebaica* Palmae. Pharm. Biol. 37 (2):114-117.

Ismail, A.E. and Eissa, M.F.M. (1993). Plant parasitic nematodes associated with ornamental palms in three botanic gardens in Egypt. Pakistan J.Nematol. 11 (1): 53-59.

Luc, M.; Sikora R.A. and Bridge J. (2005). Plant parasitic nematodes in subtropical and tropical agriculture. The 2nd edition. (eds). C.A.B. International, Oxon, UK.

Mai, W.F. and Lyon, H.H. (1975). Pictorial key to genera of plant-parasitic nematodes. 4th ed., Cornell University Press, Ithaca, New York, USA, 219 pp.

Mani, A.; Handoo, Z.A. and Livingston, S. (2005). Plant-parasitic nematodes associated with date palm trees (*Phoenix dactylifera* L.) in the Sultanate of Oman. Nematropica 35 (2):135-143.

Moawad, H. and Abd EL-Rahman, N.M. (2014) Comparison of classical and ultrasound-assisted extractions of *Hyphaene thebaica* fruit and evaluation of its extract as antibacterial activity in reducing severity of *Erwinia carotovora*. Int. Schol. Sci. Res.Innov. 86 (6): 612-614.

Norton, D.C. (1978). Ecology of plant parasitic nematodes. New York: Willey-Interscience, 268 pp.

Orwa, C, Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. (2009). Agroforestry Database: a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/treedb/AFTPDFS/Hyphaene_thebaica.pdf).

Salib, J.Y.; Michael, H.N. and Eskande, E.F. (2013). Anti-diabetic properties of flavonoid compounds isolated from *Hyphaene thebaica* epicarp on alloxan induced diabetic rats. Phcog. Res. 5 (1): 22–29.

Sasser, J.N. (1989). Plant-Parasitic Nematodes: The Farmer's Hidden Enemy. North Carolina State University Graphics, Raleigh, NC, p. 115.

Shalaby, A. and Shatta, A. (2013). Potential antioxidant and anti-inflammatory effects of *Hyphaene thebaica* in experimentally induced inflammatory bowel disease. African J.Pharmacy Pharmacol. 7 (26):1834-1840.

Sikora, R.A. and Fernández, E. (1990). Nematode parasites of vegetables. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, UK, pp. 319–392.

Täckholm, V. (1974). Students' flora of Egypt. 2nd edition published by Cairo University. Printed by Cooperative Printing Company, Beirut, Lebanon, 888 p.

Taylor, D.P. and Netscher, C. (1974). An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica 20 (2): 268-269.

Winnepenninckx, B.; Backeljau, T.and De Wachter, R. (1993). Extraction of high molecular weight DNA from molluscs. Trends in Genetics 9 (12): 407.

Youssef, M.M.A. (2014). Nematodes associated with date palm and their control measures. Arch. Phytopathol. Plant Protect. 47 (4): 425-428.

Youssef, M.M.A. and Eissa, M.F.M. (1994). Population dynamics of certain nematodes associated with date-palm in Egypt. Afro-Asian J.Nematol. 4 (1): 68-72.

Yeates, G.W. and Wouts, W.M. (1992). *Helicotylenchus* spp. (Nematoda: Tylenchida) from managed soils in New Zealand. New Zealand J. Zool. 19 (1-2): 13–23.

Zein, S.N.; Zawam, H.S. and Hassan, M.E.M. (2011). Some biochemical and molecular methods for identifying and detecting *Meloidogyne incognita*. Egypt. J.Agronematol. 10 (2): 216-230.

Zijlstra, C.; Donkers-Venne, D.T.H.M. and Fargette, M. (2000). Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology 2 (8): 847-853.

الملخص العربي

إنتشار وتوزيع النيماتودالمتطفلة والمصاحبة لأشجار نخيل الدوم في محافظة أسوان بجنوب مصر، مع التركيزعلى تعريف نيماتودا تعقد الجذور بالطرق البيوكيميائية والجُزيئية

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تم إجراء دراسة حقلية مكثفة خلال الفترة من 27 ديسمبر 2016م إلى 3 نوفمبر 2017م لحصر إنتشار أجناس النيماتودا المتطفلة والمصاحبة لأشجار نخيل الدوم في محافظة أسوان بجنوب جمهورية مصر العربية . تم لهذا الغرض جمع 105 عينة مركبة من تربة وجذور أشجار نخيل الدوم المنتشر زراعتها في كل من مركز أسوان ، دَرَاو ، إدفو وكوم أمبو التابعين لمحافظة أسوان ، وتمت عملية إستخلاص النيماتودا بطريقة المناخِل (الغرابيل) مصحوبة بالطرد المركزي والطفو في المحلول السكري . تم تعريف النيماتودا إلى مستوى الجنس تبعاً لأوصاف الجسم الأصيلة ومفاتيح التعريف القياسية للنيماتودا ، وقد أظهرت النتائج وجود أربعة عشر جنساً نيماتودياً مختلفاً في منطقة جذور أشجار نخيل الدوم ، تم ترتيبهم تنازلياً تبعاً لنسبة تواجدهم في المجموع الكلي للعينات على النحو التالي: نيماتودا تعقد الجذور *Meloidogyne* (46.7٪)، النيماتودا الكلوية *Rotylenchulus* (33.3٪)، النيماتودا الحلزونية *Helicotylenchus* (27.6٪)، نيماتودا الفطريات *Aphelenchus* (17.1٪)، نيماتودا تيلنكس *Tylenchus* (14.3٪)، النيماتودا شبه الحلقية *Hemicriconematoides* (12.4٪)، نيماتودا التقزم *Tylenchorhynchus* (9.5٪)، نيماتودا السيقان والأبصال*Ditylenchus* (8.6٪)، نيماتودا البراعم والأوراق *Aphelenchoides* (7.6٪)، نيماتودا تقرح الجذور *Pratylenchus* (6.7٪)، نيماتودا تقصف الجذور *Trichodorus* (5.7٪)، النيماتودا الحلقية *Criconemella* (4.8٪)، النيماتودا الدبوسية *Paratylenchus* (3.8٪) و النيماتودا التاجية *Hoplolaimus* (2.9٪) . تواجدت كل من نيماتودا تعقد الجذور، النيماتودا الكلوية، والنيماتودا الحلزونية بأعلى قِيَم تَمَّيز (3444 ، 2816 ، 1902 على التوالي) ، وبأعلى كثافة عددية في تلك الدراسة بلغت في المتوسط 504 ، 488 ، 362 فرداً/250 سم^3 تربة، على الترتيب ، بينما تراوحت قِيَم تَمَّيز بقية الأجناس ما بين 109-595 ، وبكثافة عددية أقل (46-201 فرداً/250 سم^3 تربة) . أكد فحص الجذور المصاحبة لعينات التربة وجود أعراض تعقد واضحة على جذور الدوم متسببة عن نيماتودا تعقد الجذور ، وقد تم تعريفها بالطرق التقليدية (إختبار البصمة الشرجية أو النمط العَجاني)، وكذا بالطرق البيوكيميائية والجُزيئية ، ليتأكد لنا أن النوع *Meloidogyne javanica* هو النوع السائد والمسبب لتلك الأعراض . قدمت تلك الدراسة تحديثاً لقاعدة البيانات والمعلومات عن النيماتودا المتطفلة على النبات وعوائلها النباتية في مصر، وذلك بإضافة نخيل الدوم عائلاً نباتياً جديداً لنيماتودا تعقد الجذور *M. javanica* وربما لبعض الأجناس الأخرى.